Eur J Clin Chem Clin Biochem
1995; 33:441–442

SHORT COMMUNICATION

Rapid and Easy Procedure for the Determination of Immunoglobulin Class and Light Chain Type of Anti-Lactate Dehydrogenase Antibodies in Macro-Lactate Dehydrogenase

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(Received February 14, 1995)

Summary: We developed an easy to perform and rapid method for determination of the immunoglobulin class and light chain type of anti-enzyme antibodies present in macro-enzymes. The procedure is a combination of two routinely used laboratory kits, and it allows identification of the antibody involved within 1.5 hour. The applicability of the method was demonstrated for macro-lactate dehydrogenase.

Introduction

Macro-enzymes are regularly seen in the clinical chemical laboratory. Generally, these are complexes of anomalous immunoglobulins bound to the enzyme, although complex formation with lipoproteins has also been described (1, 2). Immunoglobulin class and light chain type are rarely determined, since this usually requires separate time consuming procedures. Consequently, little is known about the clinical significance of differences in immunoglobulin class and light chain type in macro-enzyme complexes. We therefore developed a procedure, based on commonly applied routine clinical laboratory assays, for the rapid and easy determination of the immunoglobulin class and light chain type in macro-enzymes. In the present report the method is demonstrated in two cases of macro-lactate dehydrogenase (lactate dehydrogenase, EC 1.1.1.27).

Increased plasma lactate dehydrogenase usually indicates cell damage with ensuing leakage of the enzyme into the plasma. Another cause of increased plasma activity is the presence of macro-lactate dehydrogenases. Macro-lactate dehydrogenases are clearly revealed by isoenzyme electrophoresis, where they show diffuse and/or atypical patterns (3). The presence of macro-lactate dehydrogenases has been described in both healthy subjects and in patients with various diseases. At present macro-lactate dehydrogenases have no known diagnostic or prognostic significance, apart from the fact that their recognition saves the patient from further unnecessary investigations (4). However, differences in immunoglobulin class and light chain type of anti-lactate dehydrogenases might have significance. It has been reported that anti-lactate dehydrogenase of the IgG-class (IgG-lactate dehydrogenase) usually binds to all five isoenzymes of lactate dehydrogenase, whereas IgA-lactate dehydrogenase more specifically binds to only one isoenzyme. Most Ig-light chains in macro-lactate dehydrogenase are from the kappa-type. In particular, it has been suggested that anti-lactate dehydrogenases of the IgA-kappa type may have diagnostic or prognostic value (5).

Materials and Methods

Two patients were studied. One patient (A) was a 50-year old man with known hypertension and cardiovascular disease; his plasma lactate dehydrogenase activity concentration was 645 U/l (reference range 138–300 U/l). The other patient (B) was a 53-year old man who was seen in our out-patients clinic and screened for lactate dehydrogenase with suspected haemolytic anaemia; his plasma lactate dehydrogenase activity was 564 U/l.

Blood was collected in a heparinized tube and plasma was separated by centrifugation (3000 *g* for 10 min at 25 °C). Continuous determination of the lactate dehydrogenase activity at 37 °C was performed on a Hitachi 717 automatic analyzer (Boehringer-Mannheim), with a commercially available enzymatic method (Boehringer-Mannheim, No. 1442643).

The macro-lactate dehydrogenases were visualized and characterized by a combination of two routine techniques within one adapted procedure. The first routine technique involved the separation of lactate dehydrogenase isoenzymes by agarose gel electrophoresis (Helena Laboratories, Titan Gel LD, No. 3043). After electrophoresis the surface of the gel was gently blotted with a filter paper. This was followed by the second technique, a routine immuno-fixation procedure (Dako, Immunofixation Kit, No. K390). In short, an "antibody" template was placed over the gel, making sure that there was close contact between the template and the gel surface. The specific fixation reagents (Dako, rabbit monospecific antibodies) were placed in the troughs of the template. After incubation for 15 min at room temperature the template was carefully removed and the gel was washed (9 g/l NaCl) to remove any non-precipitated proteins. After washing and pressing the gel was incubated for 25 minutes with lactate dehydrogenase isoenzyme reagent (Helena Laboratories) at 45 °C to visualize the fixed macro-lactate dehydrogenases.

Results

The isoenzyme pattern of patient A was characterized by the absence of a typical banded pattern with the most intense lactate

dehydrogenase activity seen in a broad band in the zone between lactate dehydrogenase$_4$ and lactate dehydrogenase$_5$ (fig. 1). A haemolysate prepared from the patient's erythrocytes showed a normal lactate dehydrogenase isoenzyme pattern.

Patient B showed a lactate dehydrogenase isoenzyme pattern which was comparable to that of patient A; again no clear banded pattern was visible and the highest activity was seen in the slow zone (lactate dehydrogenase$_4$–lactate dehydrogenase$_5$) (fig. 2).

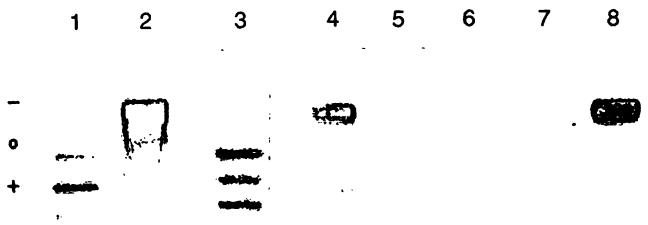

Fig. 1 Lactate dehydrogenase patterns (lane 1–3) of control plasma (lane 1), plasma from patient A (lane 2) and erythrocyte lysate (lane 3).
Immunofixation and subsequent lactate dehydrogenase staining (lane 4–8) only show visible precipitation bands after fixation with anti-IgG (lane 4) and anti-lambda light chain (lane 8).
Fixation with anti-IgA (lane 5), anti-IgM (lane 6) and anti-kappa light chain (lane 7) did not reveal bands with lactate dehydrogenase activity.

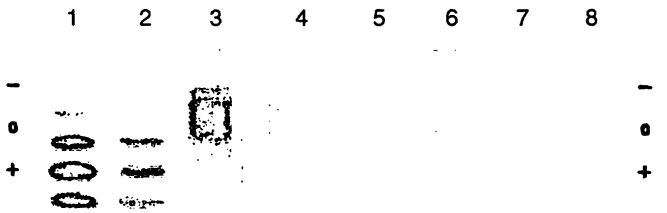

Fig. 2 Lactate dehydrogenase isoenzyme patterns (lane 1–3) of control plasma (lane 1 and 2) and of plasma from patient B (lane 3).
Immunofixation and subsequent lactate dehydrogenase-staining (lane 4–8) only show visible precipitation bands after fixation with anti-IgG (lane 4) and anti-kappa light chain (lane 7).
Fixation with anti-IgA (lane 5), anti-IgM (lane 6) and anti-lambda light chain (lane 8) did not reveal bands with lactate dehydrogenase activity.

The atypical plasma lactate dehydrogenase-isoenzyme patterns in both patients and the normal erythrocyte lactate dehydrogenase-isoenzyme pattern in patient A (not investigated for patient B) were suggestive of macro-lactate dehydrogenases. The presence of an immunoglobulin-lactate dehydrogenase complex was demonstrated by precipitating the patient sera with ammonium sulphate, then redissolving of the precipitate in normal serum. Subsequent lactate dehydrogenase-isoenzyme electrophoresis of this serum showed an atypical pattern which differed from the atypical electrophoresis pattern of the original patient serum, and which indicated the immunological nature of the complex (results not shown).

Lactate dehydrogenase-immunofixation with rabbit antibodies against IgG, IgA, IgM, kappa light chain and lambda light chain, and subsequent staining for lactate dehydrogenase activity revealed that patient A had anti-lactate dehydrogenase antibodies of the IgG-class with lambda light chains (fig. 1). Immunofixation of patient B's macro-lactate dehydrogenase showed anti-lactate dehydrogenases of the IgG-class with kappa light chains (fig. 2).

Discussion

When the laboratory results for a patient show high lactate dehydrogenase-activities which do not agree with the clinical data, the possible presence of macro-enzymes must be borne in mind. The immunoglobulin-complexed enzyme disorders are characterized by increased total serum enzyme levels that are often isolated and persistent. Confirmation of the presence of macro-lactate dehydrogenases is important, since it avoids further invasive and costly procedures to determine the cause of the increased plasma levels. Furthermore, it is possible that the lack of a specific association between macro-lactate dehydrogenases and disease is due to the limited amount of research into this topic. A further and closer identification of the anti-lactate dehydrogenases in macro-lactate dehydrogenase might reveal some new and hitherto unknown relationships between macro-lactate dehydrogenases and disease. The same could hold true for other macro-enzymes like macro-creatine kinase, macro-amylase and macro-alkaline phosphatase (4).

The present report describes a rapid and easy to perform method using a combination of two routinely used laboratory kits, which could easily be implemented in various laboratory settings. This method identifies the nature of macro-lactate dehydrogenase or other macro-enzymes within 1.5 hours. Hitherto, macro-enzyme identification was not often performed, probably because of the time-consuming and therefore costly procedures involved. Identification of the immunoglobulin-class and light chain type of the antibodies in macro-enzymes might reveal an association with certain diseased states, i.e. auto-immune disorders, malignant lesions or cardiovascular diseases, which could be useful in diagnosis.

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